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Detection of *Hp^{del}* in healthy individuals and cancer patients in Taiwan

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Abstract

Background: We investigated the genotypic distribution of *Hp^{del}* in healthy subjects and cancer patients in Taiwan.
Methods: Blood samples were collected from 244 randomly selected healthy Taiwanese volunteers and 737 patients with various cancers. Samples were analyzed for the haptoglobin (Hp) gene, and the presence of the *Hp^{del}* allele was determined from genomic DNA by an *Hp^{del}*-specific polymerase chain reaction (PCR) method. The plasma concentration of Hp was also determined.
Results: The frequency of the *Hp^{del}* allele was calculated to be 0.029, and was not different between the healthy subjects and patients with cancer. The prevalence of Hp deficiency caused by *Hp^{del}* homozygosity was estimated to be ~0.85 in 1000. Fifty-seven subjects were reclassified from homozygous *Hp^{1}* or *Hp^{2}* to *Hp^{1}/Hp^{del}* or *Hp^{2}/Hp^{del}* genotypes. The *Hp^{del}* allele is not associated with prevalence, severity or stage of any cancer.
Conclusions: Congenital Hp deficiency caused by *Hp^{del}* homozygosity is a condition present in Taiwan with a relatively high frequency. However, the *Hp^{del}* variant does not play a role in cancer.
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Keywords: cancer; Chinese; haptoglobin deficiency; haptoglobin genotype; Taiwan.

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Introduction

Haptoglobin (Hp) is a plasma glycoprotein which binds to hemoglobin to prevent iron loss and kidney damage during hemolysis. It is an acute phase protein with several functional properties, including antioxidant and anti-inflammatory activities, and regulation of the immune system. These different properties are thought to be related to polymorphisms in the *Hp* gene, which have been shown to be associated with a variety of human disorders (1, 2). An understanding of the distribution of *Hp* genotypes could help predict or prevent associated disorders, assess prognosis and enable optimal treatment.

Humans have genetic polymorphisms of Hp due to two co-dominant alleles, *Hp^{1}* and *Hp^{2}*. Combinations of these alleles result in three major phenotypes (Hp 1-1, Hp 2-1, and Hp 2-2). Recently, the *Hp*0 allele, an allele deletion in the *Hp* gene cluster, has been identified (3, 4). Hp gene analysis has shown that these individuals are homozygous for a deleted allele of the *Hp* gene, *Hp^{del}*, which is a deletion of ~28 kb in *Hp*. This deletion includes the promoter region and all of the *Hp* exons (3, 4). Interestingly, *Hp^{del}* has not been reported in some African and European-African populations (4–7), suggesting that the distribution of the *Hp^{del}* allele is limited to individuals of Asian descent, including Japanese, Koreans, Thais, and Chinese. The allele frequencies have been shown to differ among each population, varying between 1.5% and 4.4% (4–7). The distribution of *Hp^{del}* appears to be the highest, 3%–4.4%, among the Han Chinese population (5). Approximately 98% of Taiwanese are Han Chinese, but no Hp0-0-affected individuals have been found among the 172 subjects previously studied (8). Also, there have been no reports examining the allele frequency of the *Hp^{del}* variant in Taiwan. Furthermore, the allele frequency of *Hp^{del}* in the Chinese has been reported to be around 3% (5), suggesting that the *Hp^{del}* should also be high in the Taiwanese population.

There have also been many reports examining the association between the Hp phenotype (Hp 1-1, Hp 2-1, and Hp 2-2) and various cancers (1, 9, 10). One study reported on the Hp 0-0 phenotype and leukemia (11). However, association between the *Hp^{del}* allele and other forms of cancer have not been reported. We studied serum Hp and the Hp genotype in 244 randomly selected healthy subjects and 737 patients with various types of cancer to determine the *Hp^{del}* allele frequency and its relationship to cancer. The results obtained in this study will be helpful for understanding the prevalence of *Hp^{del}* among the Chinese in Taiwan, the possible frequency of congenital Hp defi-

ciency and the association of the Hp^{del} allele with various cancer.

Materials and methods

Human subjects

The study was conducted with the approval of our institutional human research Ethics Committees and was in compliance with the procedures for human studies addressed by the most recent Helsinki Declaration. Written informed consent was obtained from all patients and healthy subjects. EDTA blood samples were collected from cancer patients and healthy subjects who were selected at random. Healthy subjects were excluded if they had known inflammatory conditions such as acute or chronic infection, or major organ disease, including heart failure, renal failure or liver cirrhosis. Clinical characteristics of the cancer patient group, including clinical stage, age and gender were collected.

Hp genotyping based on polymerase chain reaction (PCR)

Genomic DNA was extracted from peripheral blood mononuclear cells using a DNA extraction kit (Qiagen, Valencia, CA, USA). The polymerase chain reaction (PCR) method for analysis of Hp^1 and Hp^2 were used as previously described (9, 12). The specimens noted to be homozygous Hp^1 (Hp^1/Hp^1) or Hp^2 (Hp^2/Hp^2) were checked by Hp^{del} genotyping. Hp^{del}-specific PCR amplification was performed with the primers, Hp-del-U and Hp-del-L, according to the methods described by Koda and co-workers (3, 4). Exon 1 of *Hp* also was amplified simultaneously with the primers, Hp-exon-U and Hp-exon-L, to detect normal *Hp*.

The oligonucleotide primers, Hp-del-U (5′-CTTTATGGCACTGGGGAACAAGCATTTTG-3′) and Hp-del-L (5′-CAGGAAGAGATTTTTAGCCGTGGTCAGCAG-3′), were used for amplification of a 315-bp Hp^{del} allele-specific sequence. The primers Hp-exon-U (5′-GCAGTGTGAAAATCCTCCAAGATAA-3′) and Hp-exon-L (5-AATTTAGCCCATTTGCCCGTTTCTT-3′), were used to amplify a 476-bp Hp1 or Hp2 allele-specific sequence. PCR was preformed in a final volume of 50 μL, containing 10–20 ng of genomic DNA. For the PCR protocol, 1X PCR buffer (Qiagen), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 mM of each primer, and two units of Qiagen *taq* DNA polymerase were included in the PCR reactions. Thermal cycler settings used for amplification were as follows: an initial incubation at 95°C for 5 min, followed by 35 cycles of incubation at 95°C for 30 s, 60°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The resulting PCR products underwent electrophoresis using a 1% agarose gel and stained with ethidium bromide. The Hp^{del}-specific PCR product was detected as a 315-bp band, while the product of the *Hp* exon 1 was detected as a 476-bp band.

Statistical analyses

Mean values and SD were calculated. Pearson's χ^2-test was used to evaluate the distribution of the three Hp genotypes among each group. A p-value <0.05 was used for statistical significance.

Results

Blood samples were collected from 244 randomly selected healthy Taiwanese volunteers and 737 patients with various types of cancer. Clinical and demographic information of patients with the more common types of cancer, including clinical stage, age and gender is shown in Table 1. The Hp^{del} allele was detected in 57 healthy subjects and cancer patients using a Hp^{del}-specific PCR method. Eleven of 94 (11.7%) subjects who were thought to have the Hp^1/Hp^1 genotype were re-classified as Hp^1/Hp^{del}. In addition, 46 of 489 (9.4%) subjects who were thought to be Hp^2/Hp^2 were reclassified as a Hp^2/Hp^{del} genotype. The frequency of the Hp^{del} allele was determined to be 0.0292. There was no difference in frequency between healthy individuals (0.0266) and those with cancer (0.0298; Table 2). The prevalence of Hp deficiency caused by Hp^{del} heterozygosity was estimated to be ~0.85 in 1000 in our cohort. The Hp^{del} allele frequencies for patients with cancer are summarized in Table 3. Patients with lymphoma had the lowest frequency of Hp^{del} (0.009), while patients in the 'other cancer' group had the highest frequency (0.041). There was no significant difference in the Hp^{del} frequency between healthy individuals and each of the different cancer groups. In addition, there were no significant difference in Hp^{del} frequency between each of the different cancer groups. We analyzed the

Table 1 Clinical characteristics of major cancer patient group.

Type	HNSCC	HCC	NPC	CRC	Breast cancer	Lung cancer	Lymphoma
Stage*							
I	3	7	0	0	6	1	7
II	17	9	2	5	25	2	9
III	13	13	7	35	18	7	15
IV	134	30	39	58	38	37	26
Total	167	69	48	98	87	47	57
Age							
Mean	54.83	65.28	50.84	35.87	51.96	63.19	62.38
SD	11.77	10.72	10.42	13.65	10.61	12.43	16.53
Gender							
Male	145	45	38	58	0	23	35
Female	22	24	10	40	87	24	22

*Cancer stage was defined as American Joint Committee on Cancer (AJCC) 6th ed., 2002. HNSCC, head and neck squamous cell carcinoma; HCC, hepatocelluar carcinoma; NPC, nasopharyngeal carcinoma; CRC, colon rectal cancer.

Table 2 *Hp* genotype prevalence in healthy controls and patients with cancer.

	Total (n=981)	Healthy group (n=244)	Cancer group (n=737)
Age (years)	50.8±12.3	51.3±14.1	50.7±11.7
Gender (M/F)	587/394	150/94	437/300
Genotype			
Hp^1/Hp^1	83 (8.5%)	18 (7.4%)	65 (8.8%)
Hp^2/Hp^1	386 (39.3%)	113 (46.3%)	293 (39.8%)
Hp^2/Hp^2	416 (42.4%)	100 (40.1%)	335 (45.5%)
Hp^1/Hp^{del}	11 (1.1%)	1 (0.4%)	10 (1.4%)
Hp^2/Hp^{del}	44 (4.5%)	12 (4.9%)	34 (4.6%)
Allele frequency			
Hp^{del}	0.0292	0.0266	0.0298
Hp^1	0.299	0.307	0.294
Hp^2	0.671	0.6659	0.676

Values are given as number (%). Odds ratio of Hp 2 vs. Hp 1, 1.063; 95% confidence interval, 0.849–1.329; p=0.594.

Table 3 Hp^{del} genotype distribution and allele frequency in healthy controls and patients with various types of cancers.

	Normal control	HNSCC	HCC	NPC	CRC	Breast cancer	Lung cancer	Leukemia	Lymphoma	Other cancer
Total allele	488	334	138	96	196	174	94	60	114	268
Hp^{del} allele	13	10	4	2	7	4	3	2	1	11
Hp^{del} frequency	0.027	0.03	0.029	0.021	0.036	0.023	0.032	0.033	0.009	0.041
p-Value*	–	0.778	0.881	0.742	0.524	0.794	0.775	0.764	0.254	0.201

*Compared with healthy group. HNSCC, head and neck squamous cell carcinoma; HCC, hepatocelluar carcinoma; NPC, nasopharyngeal carcinoma; CRC, colon rectal cancer.

Table 4 Hp^{del} genotype distribution and cancer stage* in patients with various types of cancers.

Type	HNSCC (n=167)	HCC (n=69)	NPC (n=48)	CRC (n=98)	Breast cancer (n=87)	Lung cancer (n=47)	Lymphoma (n=57)
Stage							
I/II	2 (1.2%)	2 (2.9%)	0	0	2 (2.3%)	1 (2.1%)	0
III/IV	8 (4.8%)	2 (2.9%)	2 (4.2%)	7 (9.1%)	2 (2.3%)	2 (4.2%)	1 (1.6%)
p-Value	0.353	0.258	0.910	0.673	0.488	0.115	0.778

*Cancer stage was defined as AJCC 6th ed., 2002. Values are given as number (%). HNSCC, head and neck squamous cell carcinoma; HCC, hepatocelluar carcinoma; NPC, nasopharyngeal carcinoma; CRC, colon rectal cancer.

clinical stage of the cancer patients with Hp^{del} and there was no relationship between the severity of cancer as assessed by clinical stage, and the Hp^{del} genotype (Table 4).

Discussion

Frequencies of the Hp^1 and Hp^2 phenotypes have been reported to exhibit geographic differences that are dependent on ethnicity (2). Blackwell and Thephusdin conducted a 'pheonotypic' study involving 682 healthy adults and reported that the frequency of the Hp1 phenotype among Thais was ~0.24%, and 2.3% of Thai individuals have the Hp 0-0 phenotype (13). Expression of the *Hp* gene is absent in anhaptoglobinemia (Hp 0-0 phenotype); a condition present in 1 in 1000 Caucasians. In blacks, especially of West African descent (i.e., Nigeria and Cameroon), anhaptoglobinemia is more frequent, reportedly >30% (14, 15). In the US, the frequency of Hp 0-0 in blacks is considerably less at 4% (15). Hypohaptoglobinemia has also been reported in a few non-black families carrying a ''silent allele'' with no gene product (Hp0 phenotype; 2). Hp 0-0 has also been reported in Asians. However, it should be noted that a complete genetic model has not yet been reported for the Hp0 phenotype in populations other than Asians [Hp^{del} genotype; 3, 5, 6, (16)]. Teye et al. reported that the A–61C mutation in the promoter of the *Hp* gene and another mutation in exon 7 in the β-chain of the Hp^2 allele reduced transcription activity and Hp gene expression (17, 18). In addition, these mutations seem to be related to some of the anhaptoglobinemic individuals in Africa (19). No other detectable change in the *Hp* gene clusters, including the promoter region, of Hp0 individuals has been reported in other areas. Some authors presume that the reduced expression of Hp is related to the trans-acting factors necessary for *Hp* expression, or cis-acting promoter sequences (20). However, at present there is no apparent research to confirm the speculation in other areas. Blackwell et al. (8) also

investigated the Hp phenotype among Taiwanese. Unfortunately, their method was not a genetic survey by PCR but rather a genotypic survey by electrophoresis. Thus, the individuals with the heterozygous allele of Hp0 (Hp1-0 and Hp 2-0) could be misidentified as Hp 1-1 or Hp 2-2. In our series, the *Hp*^{1} allele frequency (33%) was nearly the same as previously reported by Blackwell (28%). However, the Hp0 frequency as determined by the PCR method was more accurate in detecting individuals with Hp0 heterozygosity. Nevertheless, differentiating between congenital and acquired hypohaptoglobinemia could be difficult. There are many factors that may interfere with the diagnosis of hypohaptoglobinemia, including age, underlying congenital diseases such as hemolytic disorders (e.g., hereditary red cell membrane and enzyme defects, thalassemia, and sickle cell anemia), and even lifestyle factors (e.g., repetitive physical exercise associated with limited mechanical trauma to erythrocytes). No Hp can be detected in neonatal serum. However, by the sixth month of life, failure to detect Hp becomes relatively rare. About 75% of Hp0 subjects with *Plasmodium vivax* infection, when treated with chloroquine, showed typable Hp polymorphs by 8–9 days post-treatment (21). The *Hp*^{del} variant has been detected exclusively in Asians, thus it may represent the most important cause of Hp0 in this population (5, 19). Therefore, we suggest a genetic survey as an easier and more accurate method to detect the Hp0 genotype in the Asians.

Some case reports have examined congenital Hp deficiency among cases of severe anaphylactic transfusion reactions (22–24). All of these cases occurred in Japanese who were homozygous for a deleted allele of the *Hp* gene, *Hp*^{del}. Alloimmunization may play a role in the development of anaphylactic nonhemolytic transfusion reactions due to the presence of both IgG and IgE class anti-haptoglobins, which have been noted in patient blood (22–24). Transfusion of Hp-depleted blood products should be considered in order to avoid such reactions (25). The frequency of the *Hp*^{del} allele among the Taiwanese population was determined to be 0.03 and the prevalence of Hp deficiency was estimated to be as high as 1 in 1000. However, there have been no case reports describing congenital Hp deficiency among the Chinese. This may be due to misdiagnosis of many cases of anhaptoglobin physicians. Patients are not identified as Hp-deficient until they experience anaphylactic transfusion reactions during the course of treatment for their diseases, and healthy volunteer donors with Hp deficiency do not show any pathogenic tendencies. We have begun a study to survey *Hp*^{del} frequency in patients suffering from severe transfusion reactions in our hospital. In addition, congenital Hp deficiency should be one of the major causes of severe transfusion reactions among Chinese as well as other Asians. In addition, transfusion of Hp-depleted blood products should be effective in avoiding such reactions. We stress that congenital Hp deficiency is an important risk factor for transfusion in Chinese.

Serum Hp levels have been reported to be lower in the subjects of Hp 2-2 than in Hp 1-1 and Hp 2-1 (2, 7, 26). In particular, the *Hp*^{2}/*Hp*^{del} genotype was reported to be associated with an abnormally low Hp concentration (3, 6, 7). The physiologic explanation for this condition needs to be investigated further. It has been suggested these patients may have a lower clearance rate for heme and other super-oxidant substances because of lower Hp levels. This may interfere with the clinical course of their disease. Although many studies have reported the biological or clinical importance of Hp in various diseases, including cardiovascular disease, diabetes, or cancer (2, 27), congenital Hp deficiency does not appear to be associated with any pathologic conditions other than transfusion reactions. Different biological activity has been described to account for differences between Hp^{1} and Hp^{2}. These include differences in hemoglobin-binding ability, protection against free radicals, inhibition of prostaglandin synthesis, and bacteriostatic effects. In our study, individuals with the *Hp*^{del} allele did not have a higher incidence of cancer, and *Hp*^{del} does not appear to play a role in carcinogenesis. Further study of the patients with *Hp*^{2}/*Hp*^{del} should be done to clarify whether lower Hp levels worsen prognosis.

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